73 SiRNA calnexin promotes endogenous F508del-CFTR trafficking

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The most common mutation in cystic fibrosis (CF), F508del, results in CFTR (CF transmembrane conductance regulator) protein that is retained in the endoplasmic reticulum. Previously, we have shown that miglustat corrects the trafficking of F508del-CFTR and hypothesized that by inhibiting the interaction of F508del-CFTR with calnexin, miglustat prevents its retention and degradation (Norez et al., 2006). However, others contest the role of calnexin in the F508del-CFTR retention (Chang et al., 2008). The purpose of this study was (i) to determine the effect of Small interfering RNA (SiRNA) calnexin transfection on endogenous F508del-CFTR trafficking, (ii) to compare these results with a miglustat-induced correction, (iii) to understand whether calnexin is implicated in the F508del-CFTR trafficking. SiRNA calnexin transfection was done on the CF-KM4 tracheal cell line to inhibit expression of calnexin. The level of calnexin expression was verified by biochemical technique and consequences on CFTR and ENaC activities were assessed using single-cell fluorescence imaging. We demonstrated that decreasing calnexin expression restores F508del-CFTR activity at the plasma membrane. This correction was associated with a decrease of ENaC activity. Surprisingly, we found a higher level of correction induced by miglustat than the one of siRNA calnexin, suggesting that inhibition of calnexin/F508del-CFTR interaction is probably not sufficient to fully explain the effects of miglustat raising the hypothesis that another molecular target for this drug exists.
In conclusion, this work is in favor of a role of calnexin in the F508del-CFTR retention and confirms calnexin as a valuable CF therapeutic target.
Supported by Vaincre la Mucoviscidose and CNRS.

74* Agonists of peroxisome proliferator activated receptor gamma (PPARγ) modulate transepithelial anion secretion in human bronchial epithelial cells

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The recognition that peroxisome proliferator activated receptors (PPARs) are regulators of metabolic and inflammatory pathways has led to the development and use of agonists of these receptors as therapeutic agents for wide variety of diseases. Three different PPAR subtypes have been identified: PPARα, PPARβ/δ, and PPARγ. In this study we examined the role of PPARγ agonists in human airway transepithelial ion transport. We found that PPARγ regulated both the cAMP and Ca^{2+} signaling pathways. Forskolin and carbachol responses were examined to determine ionic contributions to the decline in short circuit current (SCC) after PPARγ agonist treatment. The reduction in forskolin response was due to decreased bicarbonate secretion, while the decrease in carbachol SCC was due to reduced chloride secretion. ATP responses were modulated at both, apical and basolateral membranes by PPARγ agonists. While apical ATP SCC responses were increased, basolateral ATP responses were decreased after PPARγ agonist treatment. This difference appears to be the result of increased apical calcium-dependent secretion for the apical membrane response and decreased calcium dependent secretion for the basolateral ATP response. The effect of PPARγ on the expression of ion channels and transporters was also examined. PPARγ agonists regulated the expression of NBC1 and CFTR. PPARγ increased CFTR promoter activity in gene reporter assays and increased CFTR mRNA detected by PCR. However, functional and western blot data showed that CFTR expression was not increased. This suggests that while CFTR is regulated by PPARγ, homeostatic mechanisms prevent the overexpression of CFTR in human bronchial epithelial cells.
Supported by: Studies supported by the Canadian Cystic Fibrosis Foundation.

75* Connexin 26 is implied in the regulation of airway epithelium repair

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Functional integrity of the airway epithelium is altered in cystic fibrosis (CF). Epithelial integrity depends on the expression and assembly of specific proteins into specialized junctional structures. Gap junctions, made of connexins (Cx) hexamer, play crucial roles in these interactions by contributing to the ability of cells to share signaling factors directly between adjacent cells. The pattern of Cx expression in Human Airway Epithelial Cell (HAEC) cultures is associated with the differentiation state. Thus, Cx26 is specifically expressed during proliferation phase and its expression decreases to undetectable level with HAEC differentiation to a polarized airway epithelium. In a model of HAEC repair, where the cultures are mechanically wounded, Cx26 is transiently re-expressed at the wound area and in basal cells behind the wound. The re-expression of Cx26 is associated with enhanced spreading of the gap junction tracer Lucifer Yellow. In normal HAEC, Cx26 detection is concomitant with the cell ability to proliferate, as evaluated by Ki-67 detection, to close the gap following injury. The same phenomenon is seen in HAEC from CF patients in higher proportion. Interestingly, the amount of Cx26, the duration of its expression and the number of Ki-67-positive cells are amplified, suggesting a hyperproliferative state of the CF airway epithelium. The use of gap junction blockers delays the epithelial repair and targeting Cx26 with a specific siRNA reduced HAEC proliferation. These results suggest that gap junctions, and more specifically Cx26, play a role in airway epithelium wound repair and that understanding of the underlying mechanisms may lead to identify new targets for controlling CF HAEC proliferation and differentiation.
Supported by: “Vaincre la mucoviscidose” and FNRS.

76* Connexin channels mediate PGE2-dependent regulation of CFTR activity in Calu-3 cells

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Connexins form hemichannels and gap junction channels enabling for extracellular and intercellular coordination of tissue activity. In airway epithelial cells, CFTR activity is regulated by protease-activated receptors (PAR) at the basolateral membranes and adenosine (ADO) receptors at the apical membrane; both pathways require the release of PGE2 to stimulate basolateral EP receptors and activate CFTR. Interestingly, the extent of gap juctional intercellular communication (GJIC) was modulated by ADO pathway in Calu-3 cells. ADO is produced by hydrolysis of extracellular nucleotides by the membrane-bound ectoenzyme CD73. CD73 activity in Calu-3 cells could be increased by stimulation with a methotrexate analogue or inhibited by the CD73 blocker AMP-CP. Stimulation and inhibition of CD73 activity enhanced and decreased GJIC, respectively, through ADO release. In addition, inhibition of phospholipase A2 and cyclooxygenase prevented increase in GJIC in response to the ADO receptor agonist NECA, suggesting for a role of PGE2 in this regulation. Indeed, PGE2 was also found to markedly increase GJIC in Calu-3 cells. PAR-dependent activation of CFTR was dependent of GJIC. Thus, reduction of GJIC with AMP-CP, a gap junction blocker and a specific connexin mimetic peptide prevented CFTR currents induced by PAR in Ussing chambers. CFTR currents were also inhibited with antagonists of EP2 and EP4 receptors, again involving PGE2 in this mechanism. We further show that connexins form hemichannels at the basolateral membrane. Work is in progress to test the hypothesis that PGE2 release by hemichannels contribute to the regulation of CFTR activity via cAMP intercellular propagation through gap junction channels.
Supported by: VLM, FNRS, Schmidheiny and Novartis Consumer Health.